Ion Trap Tandem Mass Spectrometry Study of Dexamethasone Transformation Products on Light Activated TiO_2 Surface

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The photocatalytic transformation of dexamethasone and the formation of its intermediate compounds have been studied using titanium dioxide as a photocatalyst. The degradation of dexamethasone occurs easily through the formation of several hydroxy derivatives whose characterization has been made by HPLC/MS/MS. Even if both oxidative and reductive processes can be operating, only oxidative products have been identified in air saturated aqueous suspensions. A pattern of reaction pathways accounting for the observed intermediates is proposed. The obtained experimental evidence may be rationalized postulating the existence of a double initial mechanism. A single oxidation step resulting from the attack by one ·OH radical leading to the formation of five hydroxy-derivatives and a concomitant attack involving two ·OH radicals leading to the hydroxylation of the quinoid moiety of the molecule. (J Am Soc Mass Spectrom 2001, 12, 1286–1295) © 2001 American Society for Mass Spectrometry

Dexamethasone is a synthetic analog of corticosteroid hormones having several specific pharmacological effects widely used for treatment of inflammation, allergy, and diseases related to adrenal cortex insufficiency. However, in suitable administration conditions this may have anabolic properties and as such is frequently used illegally to contribute to the alteration of the characteristics of meat in beef cattle by increasing the lean–fat ratio. The use of this substance alone or in combination with other synthetic corticosteroids for growth promoting properties in livestock presents several problems for regulatory agencies because its elimination from urine and plasma leaves very low residue levels for monitoring in tissues.

It may be very useful to be able to identify the possible product degradation of dexamethasone in order to research it along with the parent compound in real samples. At present only a few metabolic in vitro studies in which dexamethasone is metabolized to 6-hydroxydexamethasone and side-chain cleaved metabolites are available [1–3]. A detailed metabolic study could provide useful information in indicating suitable analytes upon which to better base confirmatory analysis procedure. However, some information about this aspect may be obtained adopting a photocatalytic degradation system which in several cases may provide a satisfactory simulation of the metabolic transformations occurring in living organisms.

Photocatalytic degradation with irradiated semiconductors [4–11] has been established to be effective for degradation and final mineralization of several organic compounds. Heterogeneous photocatalytic processes involve reactions at the surface/solution interface [12]. The oxidizing species may be holes (h^-_{VB}, more oxidizing than aqueous hydroxy radical) or trapped holes (less oxidizing than aqueous hydroxy radical) [13]; the reducing species may be conduction band electrons (e^-_{CB}) or trapped electrons, both less reducing than aquated electrons [14–16]. Whether a process takes place under photocatalytic conditions is related to the redox property of e^-_{CB} and h^-_{VB} on the one part and dexamethasone on the other.

Considering that oxidative and reductive photocatalytic processes are operating and that the presence of oxygen adds further and alternative degradation pathways, we have also investigated the photocatalytic transformations of dexamethasone in the presence of a hole scavenger to estimate the importance of the reductive pathways. An accurate analysis of the MS/MS spectra has been made in order to confirm the nature of the intermediate structures.

Experimental

Material and Reagents

All experiments were carried out using TiO_2 Degussa P25 as the photocatalyst. In order to avoid possible interferences from ions adsorbed on the photocatalyst, the TiO_2 powder was irradiated and washed with

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distilled water until no signal due to chloride, sulfate, or sodium ions could be detected by ion chromatography.

Dexamethasone (Aldrich, Milan, Italy) was used as received. HPLC grade water was obtained from MilliQ System Academie (Waters, Millipore, Milford, USA). Methanol HPLC grade (BDH, Poole, England) was filtered through a 0.45 μm filter before use. Ammonium acetate reagent grade was purchased from Fluka Chemie (Sigma, Milan, Italy).

Irradiation Procedures

The irradiations have been performed using a 1500 W xenon lamp (Solarbox, CO.FO.MEGRA, Milan, Italy) simulating AM1 solar light and equipped with a 340 nm cutoff filter. The irradiance spectrum and the cells were identical to those described elsewhere [17, 18]. The total photonic flux (340–400 nm) in the cell and the temperature during irradiation have been kept constant for all experiments. They were 1.35×10^{-5} einstein min^{-1} and 50 °C, respectively. The irradiation was carried out on 5 ml of suspension containing 10 mgL^{-1} dexamethasone and 200 mgL^{-1} TiO_2. The entire content of the cells was filtered through a 0.45 μm filter and then analyzed by an HPLC-MS instrument.

Analytical Procedures

Liquid chromatography The chromatographic separations were run on a C18 column Inertsil5 ODS3, 250 × 4.6 mm (Chrompack, The Netherlands). Injection volume was 100 μL and the flow rate was 1.0 mL/min. Isocratic mobile phase composition was adopted: 65/35 methanol/aqueous ammonium acetate 20 mM pH 6.8.

Mass spectrometry A LCQ MAT ion trap mass spectrometer (ThermoFinnigan, Bremen, Germany) equipped with an atmospheric pressure interface and an APCI ion source was used. The LC column effluent was delivered into the ion source through a heated nebulizer probe (400 °C) using nitrogen as sheath and auxiliary gas (Claind Nitrogen Generator apparatus, Milan, Italy). The corona discharge voltage was set at the 5 kV value. The heated capillary value was maintained at 220 °C. Positive ions were acquired in full scan mode. The acquisition method used was previously optimized in the tuning sections for the parent compound (capillary, magnetic lenses, and collimating octapoles voltages) in order to achieve maximum sensitivity.

Results and Discussion

Photogeneration of Active Surface Species

Surface hydroxyl radicals generated by water oxidation according to the reactions (eq 1–4) reported below, yield to the formation of species active in the photodestruction of the organic compounds:

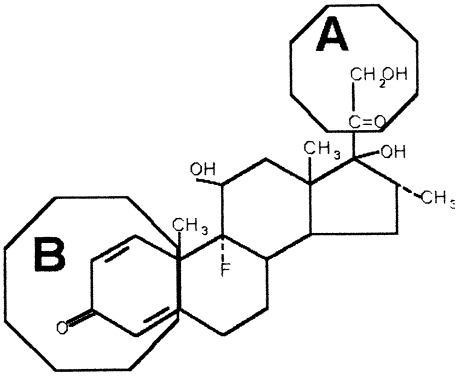


Figure 1. Molecular structure of the dexamethasone. The more interesting zones of reaction are indicated in the molecule with A and B.

$$TiO_2 + h\nu \rightarrow e^-_{CB} + h^-_{VB} \quad (1)$$

$$H_2O + h^-_{VB} + \rightarrow H_2O\cdot^- \quad (2)$$

$$H_2O\cdot^- \rightarrow \cdot OH_{(surf)} + H^- \quad (3)$$

$$2H^- + 2e^-_{CB} + O_2 \rightarrow H_2O_2 \quad (4)$$

Hydroxy radical (HO·) is a reactive, nonselective species [19, 20]. Hydroxyl reacts rapidly and nonselectively with most organic compounds by H-abstraction and addition to C–C unsatured bonds [21, 22]. Surface peroxides [23–25] can be formed by hydroxy radical pairing (hole pairing) through reactions (eq 5 and 6):

$$2(OH)_{surf} \rightarrow (H_2O_2)_{surf} \quad (5)$$

$$(H_2O_2)_{surf} \rightarrow (O_2^{2-})_{surf} + 2H^- \quad (6)$$

In the presence of air, conduction band electrons can likewise reduce oxygen forming superoxide radicals (eq 7). These superoxide radicals, which are known to strongly adsorb to the TiO_2 surface, dismutate in neutral (or acidic) solution into O_2 and peroxide according to eq 8:

$$e^-_{CB} + O_2 \rightarrow O_2^-. \quad (7)$$

$$2(O_2^-)._{surf} \rightarrow O_2 + (O_2^{2-})_{surf} \quad (8)$$

Compared to ·OH radical, $HO\cdot_2$ is much less reactive [26] and its conjugate base $O\cdot_2^-$ (pK_a, 4.8) is practically unreactive as a free radical [27]. The role played by these species on the dexamethasone degradation will be discussed below.

Photoinduced Transformation of Dexamethasone

The disappearance of dexamethasone (see molecular structure in Figure 1 and MS/MS spectrum in Table 1)

Table 1. Fragmentation obtained from MS/MS spectra of dexamethasone *m/z* 393 ($C_{22}H_{29}O_5F$)

Fragment	Relative intensity	Possible structure	Associated loss
393	2	$C_{22}H_{29}O_5F$	-
373	100	$C_{22}H_{28}O_5$	HF
355	38	$C_{22}H_{26}O_4$	H_2O
337	15	$C_{22}H_{24}O_3$	H_2O
325	5	$C_{21}H_{24}O_3$	CH_2O
297	4	$C_{20}H_{24}O_2$	CO

and the evolution of the intermediates have been accurately monitored by HPLC-MS analysis of solutions at various times of degradation. The peculiar information extractable from the MS/MS spectrum are reported in Table 1. The main fragment is the result of the breaking up of the C–F bond with release of a HF molecule. Subsequently, two molecules of water (coming from the alcoholic groups) are released. A concomitant fragmentation pathway involves part A of the molecule and results in the fragment *m/z* 325 which comes from *m/z* 355, after a formaldehyde loss. It is then followed by a further loss of CO (the fragment holding *m/z* 297).

The kinetics of dexamethasone (2.5×10^{-5}M) disappearance is represented in Figure 2, showing the cases in which the compound is in pure water or in a solution containing methanol 0.64 M. The dexamethasone degradation in pure water occurs easily, whereas the presence of methanol strongly decreases the degradation rate (the half-life time passes from 2 to 70 min). Methanol acts as a hole scavenger, being an organic electron donor, and is able to react with both free and adsorbed ·OH radical [28, 29]. It disturbs the equilibrium between the photogenerated e^- and h^- pairs, thus favoring the availability of reductive species. The observed inhibition suggests that dexamethasone can easily be oxidized and that its photodegradation proceeds through oxidative pathways rather than through photogenerated electrons in an air equilibrated system. This result stresses the importance of oxidative attacks. In metabolic studies carried out in liver, 6-hydroxydexamethasone, 6-hydroxy-9 α-fluoro-androsta-1,4-diene-11-β-hydroxy-16-alphamethyl-3,17-dione(6-hydroxy-9-αF-A) have been identified [1]. These structures could similarly be obtained through an oxidative attack mediated by ·OH radical. In these terms, the photocatalytic method can be used as a way to simulate the oxidation reactions occurring in animal liver.

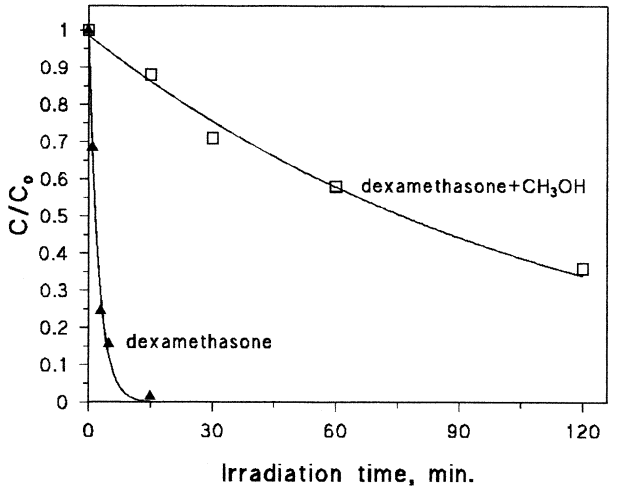


Figure 2. Disappearance of dexamethasone (2.5×10^{-5}M) on TiO_2 200 mgL^{-1}; alone and in presence of methanol 0.64 M.

Intermediates Evolution

From the previously described model of catalyst surface species photogeneration, mainly oxidation products are expected. Dexamethasone at a higher concentration (1.3×10^{-4}M) has been degraded in order to better follow the evolution of intermediates and to easily perform the MS/MS spectra analysis; in these experimental conditions, the half-life for dexamethasone corresponds to 8 min.

Figure 3 shows the obtained chromatographic profile of the solution at 5.0 min of degradation as an example. As it can be seen, numerous species have been identified characterized by different *m/z* ratios along with several peaks corresponding to an equal *m/z* value. The evolution of the different peaks in function of the irradiation time is reported in Figures 4, 6, 7, and 8. It can be seen that the compounds having *m/z* 409 and 425 are initially formed in a relevant amount suggesting that they are the main products of the early transformation steps of dexamethasone. Their simultaneous formation can therefore be considered as proof of their production through two different mechanisms.

On the basis of the obtained experimental evidence, two possible mechanisms of initial dexamethasone transformation are shown in Scheme **1** and Scheme **2**, taking into account the identified intermediates, their kinetic evolution, and their distribution. From such schemes emerge the findings that transformation proceeds essentially through an oxidative process, the active species of which is hypothesized to be the surface bound ·OH radical. The first pathway passes through the formation of the species at *m/z* 409, while the second one passes through the initial formation of the species at *m/z* 425.

Looking closely at the first pathway, five peaks have been detected with *m/z* 409, the formation and disappearance kinetics of which are shown in Figure 4. More than one derivative is simply the consequence of the nonselectivity of the ·OH radical attack. Carbon centered radicals generated by hydroxyl radical attacks may react with O_2 to give organoperoxy radicals (ROO·) which can decompose to form HO_2, or ultimately, nonradical oxygenated products [30, 31]. All of them present retention times shorter than dexamethasone. Their MS/MS spectra analyses are represented in Table 2. Comparing the fragments of these species with those coming from dexamethasone, several analogies come

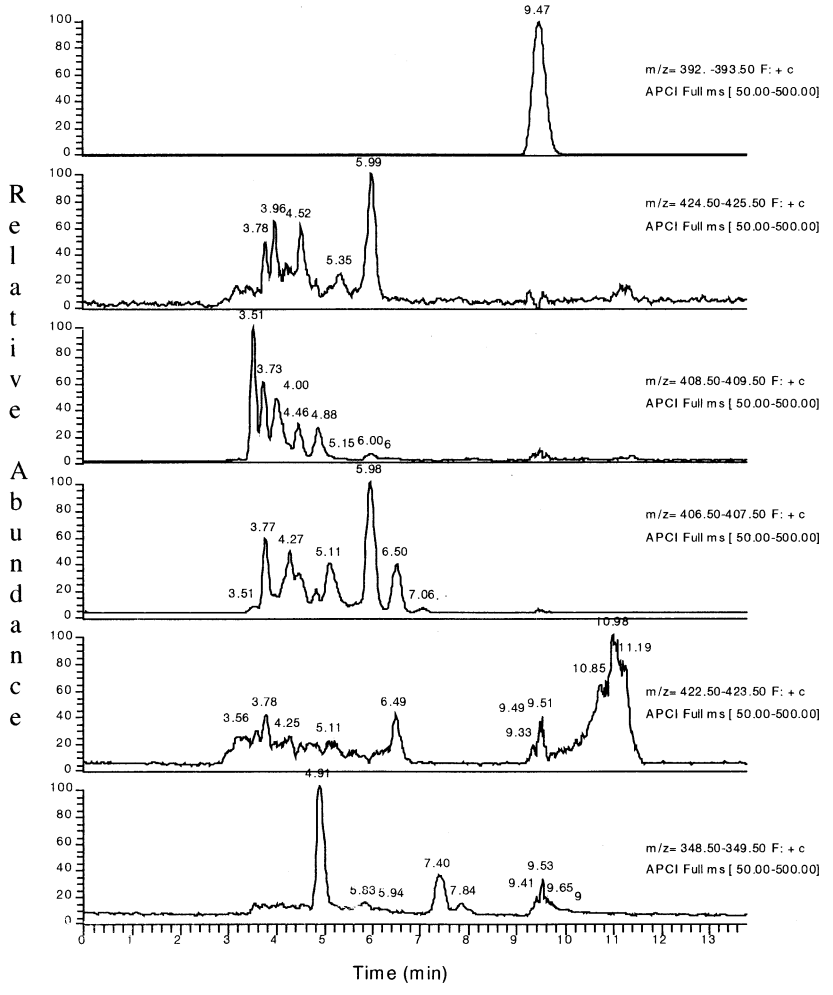


Figure 3. Chromatographic profile of the solution at 5.0 min of degradation.

up. The only difference is an additional loss of H_2O molecule probably linked to the presence of one more alcoholic group. In fact, their MS/MS analysis clearly shows the loss of three H_2O molecules, one more than the parent molecule. It would seem reasonable to assign them the structure of the hydroxylated dexamethasone. From the MS/MS evidence we conclude that hydroxylation occurs on the methylene groups with the formation of the intermediates shown. Scheme **1** shows the suggested structures having *m/z* 409 labeled **1** to **5**. We assume that the formation of 6-hydroxydexamethasone already found in liver studies [1–3], and 7-, 8-, 14-, and 15-hydroxydexamethasone, are evident.

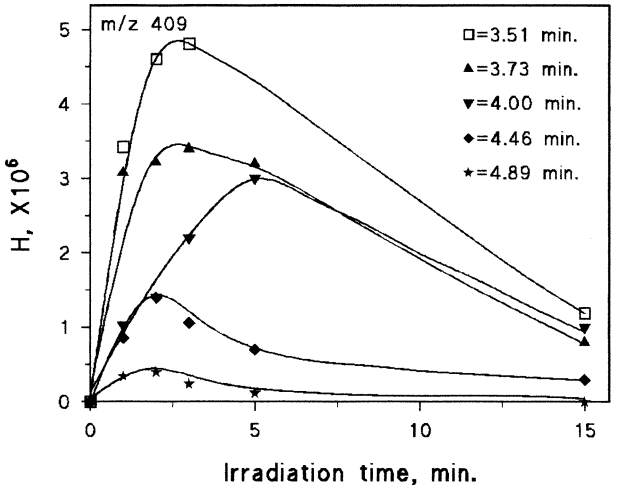
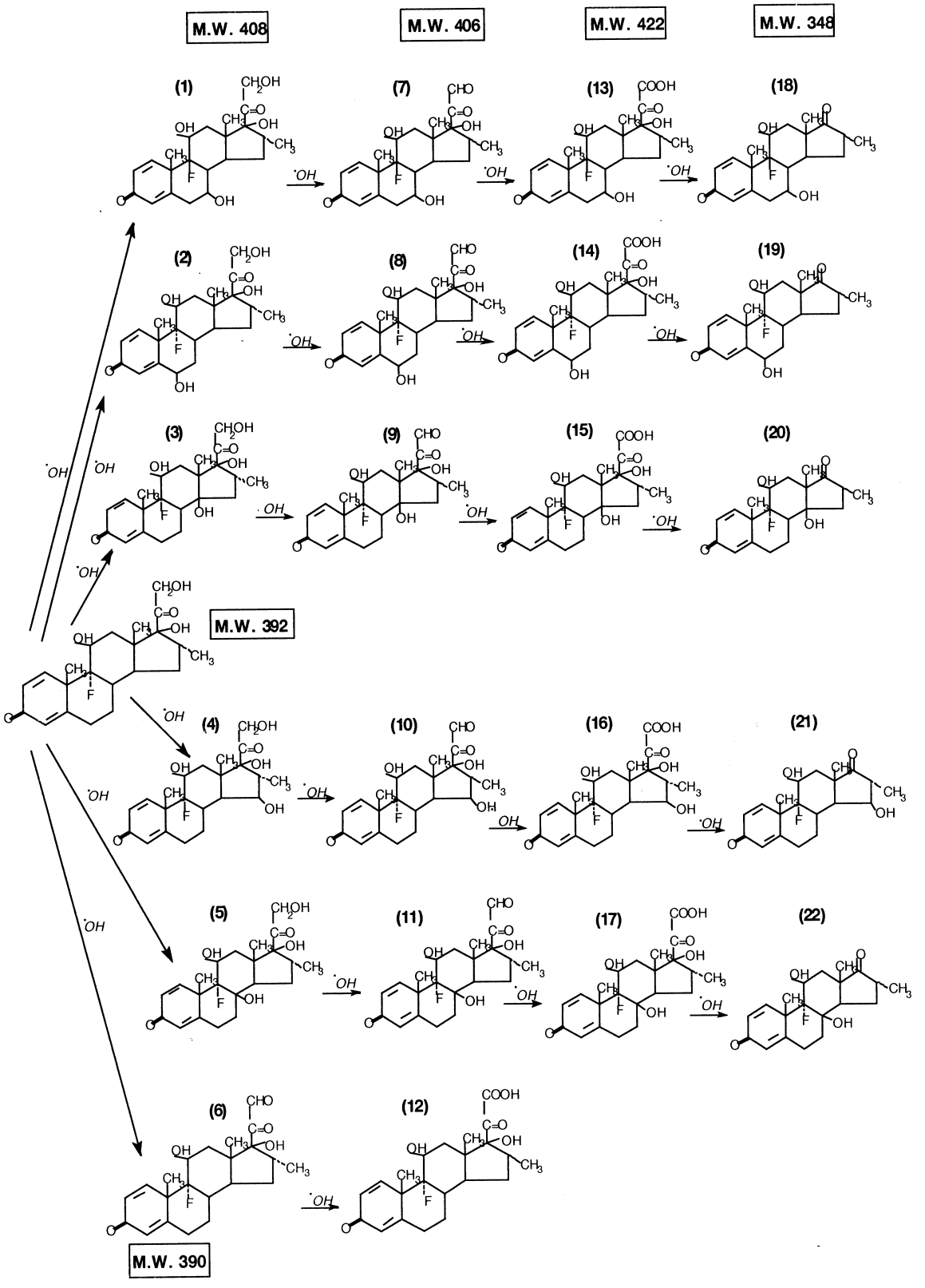


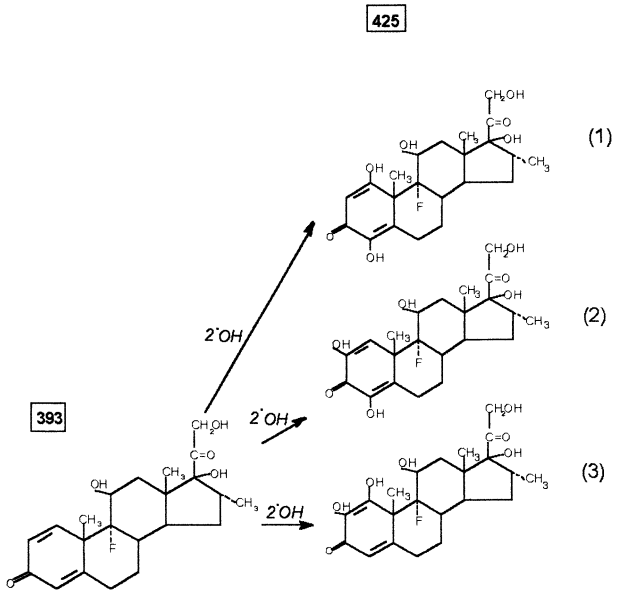
Figure 4. Formation of intermediates from dexamethasone (1.3×10^{-4}M) degradation on TiO_2 200 mgL^{-1}; *m/z* 409.

They all fragment in the same manner but with different relative intensities of the ions. While in the dexamethasone spectrum HF is the favorite loss, with *m/z* 409 it is still true on spectra **1**, **2**, and 5, but no more on spectra **3** and **4**. This is probably due to the possibility to form a stable system, not able to originate in the case of dexamethasone. This can be seen to happen peculiarly in dependence of the extension of the conjugated system induced by the concerted losses of the HF molecule and of the H_2O molecules. The two examples of fragmentation scheme reported in Figure 5 illustrate the difference in daughter ions conjugation in depen-

Scheme 1



Scheme 1 Initial transformation pathways involved in the degradation of dexamethasone; one OH radical attack.



Scheme 2 Initial transformation pathways involved in the degradation of dexamethasone; double initial OH radical attack.

dence of the position of hydroxylation. The fragmentation of type 1, similar for the structures labeled **1** and **2** in Scheme **1**, leads to the formation of molecules with an extended conjugated system. Spectra and chromatograms which are too similar do not permit the distinction between these two species. The fragmentation of type 2 occurs on molecules with structures like **3**, **4**, and **5** (see Scheme **1**) and leads to fragments having a less conjugated system.

Concurrently, the formation of molecules having *m/z* 407 with retention times slightly increased in respect to species at *m/z* 409 (Figure 3) can be seen. Such experimental evidence is in accord with the oxidation of the alcoholic group into an aldehydic one. Several species at *m/z* 407 are present, all of which come from the oxidation of the hydroxylated structures previously described, except for the one coming from the pathway **6** (Scheme **1**) leading to the formation of the carboxylic derivative.

Although not represented in Scheme **1**, the species having *m/z* 407 labeled 7–11 can also be formed from the

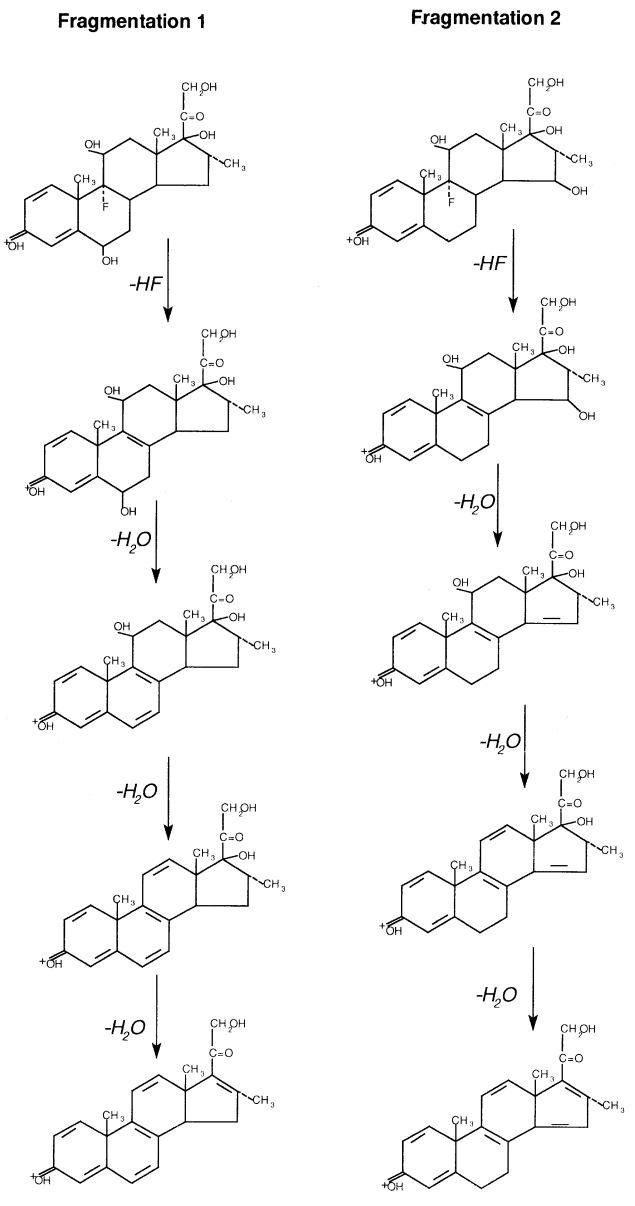


Figure 5. Fragmentation scheme for the different species having *m/z* 409.

Table 2. Fragmentation obtained from MS/MS spectra of species holding *m/z* 409 ($C_{22}H_{29}O_6F$)

Fragment	Possible structure	Associated loss	1. t_R 3.51 min, intensity	2. t_R 3.73 min, intensity	3. t_R 4.00 min, intensity	4. t_R 4.46 min, intensity	5. t_R 4.89 min, intensity
389	$C_{22}H_{28}O_6$	HF	100	100	22	60	100
371	$C_{22}H_{26}O_5$	H_2O	40	58	100	100	85
353	$C_{22}H_{24}O_4$	H_2O	28	32	18	40	16
335	$C_{22}H_{22}O_3$	H_2O	15	21	15	20	5
307	$C_{21}H_{20}O$	CO	8	10	5	10	3
323	$C_{21}H_{22}O_3$	CH_2O	5	6	5	5	5
295	$C_{20}H_{22}O_2$	CO	5	5	5	5	3

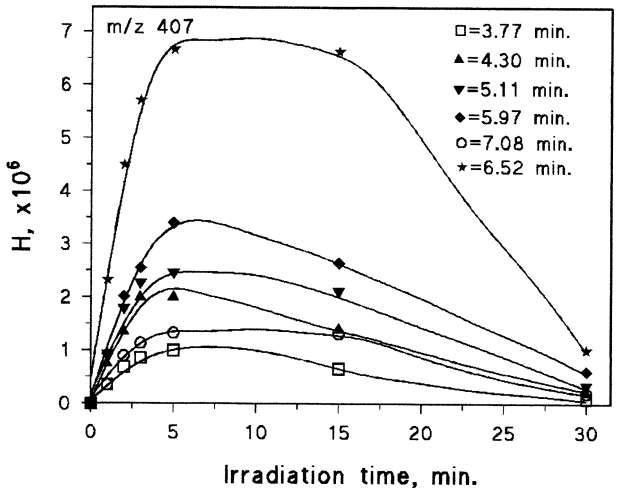
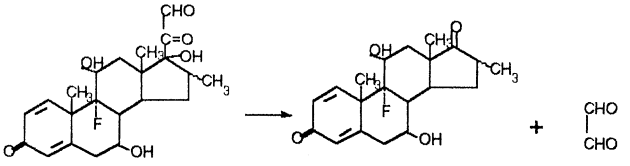


Figure 6. Formation of intermediates from dexamethasone (1.3×10^{-4}M) degradation on TiO_2 200 mgL^{-1}; *m/z* 407.

species *m/z* 391, as an alternative to the oxidation of the aldehydic group. Furthermore, this type of oxidation occurs rapidly, as results by comparing the evolution kinetics of the intermediates reported in Figures 4 and 6 with evolution times only slightly delayed for the *m/z* 407 species.

The fragmentation pattern obtained from MS/MS analysis (Table 3) completely supports the proposed structure. In fact, even though fragmentation details are basically analogous to those of species at *m/z* 409, there nevertheless is an important difference because of the presence of the ions at *m/z* 323, 329 and 305. Also, in this case the intensity of the same *m/z* values for the various daughter ions differs because of the variation in stability depending on the substitution position of ·OH groups. A loss of the CO molecule obtained through several fragmentation pathways has also been observed. The ions at *m/z* 323 and 305 are 28 aμ distant from the ion at *m/z* 351 and 333 and are the result of the loss of the CO molecule. This indicates the presence of a carbonilic group. Moreover, the loss of a glyoxal molecule (fragment holding *m/z* 329) is very peculiar and leads to the following molecule:



This fragment is seen in species 7 to 11 (Scheme **1**). A further confirmation of the involvement of part A of the molecule comes from the absence of formaldehyde loss, a fragment present both on dexamethasone and *m/z* 409 spectra.

From the MS/MS analysis of the spectrum labeled **6**, some interesting differences emerge. The peculiar losses evidenced in spectra **1** to **5** are not present anymore. Instead of this, concomitantly to the fragmentation through HF and H_2O losses, a fragment resulting from the CO_2 loss (*m/z* 345) has been identified, thereby underlining the presence of a carboxylic group. This species is also the least hydrophilic molecule among the six presented above, i.e., it contains an OH group which is less than the others. These considerations lead us to believe that spectrum **6** can be attributed to the structure labeled **12** in Scheme **1**.

The degradation process can further go on through the oxidation of the aldehydic group to the correspondent carboxylic acid derivative with a change of *m/z* value from 407 to 423 (Figure 7). More than one species gave a response in MS/MS analysis (Table 4), showing however, a weak signal as a result of the difficulty in seeing the carboxylic acids in positive ions detection mode. Even if an initial loss of HF is seen in all cases, the typical fragmentation of an acid consequently follows. A fragment holding *m/z* 359 is present in all the mentioned structures because of the acid decarboxilation. This clearly emerges from spectra **1** to **4**. In spectrum **5** however, fragmentation is very confusing because of a low signal, and it is hardly interpretable.

Table 3. Fragmentation obtained from MS/MS spectra of species holding *m/z* 407 ($C_{22}H_{27}O_6F$)

Fragment	Possible structure	Associated loss	1. t_R 3.81 min, intensity	2. t_R 5.1 min, intensity	3. t_R 6.52 min, intensity	4. t_R 4.3 min, intensity	5. t_R 5.78 min, intensity	6. t_R 7.08 min, intensity
387	$C_{22}H_{26}O_6$	HF	78	25	100	100	100	100
369	$C_{22}H_{24}O_5$	H_2O	100	100	55	76	85	69
351	$C_{22}H_{22}O_4$	H_2O	33	38	9	33	60	30
333	$C_{22}H_{20}O_3$	H_2O	17	8	3	20	22	3
323	$C_{21}H_{22}O_3$	CO	19	16	3	11	20	-
329	$C_{20}H_{24}O_4$	$C_2H_2O_2$	12	10		5	4	-
305	$C_{21}H_{20}O_2$	CO	7	30	3	9	23	-
345	$C_{21}H_{25}O_3F$	CO_2	-	-	-	-	-	50
325	$C_{21}H_{24}O_3$	HF	-	-	-	-	-	3

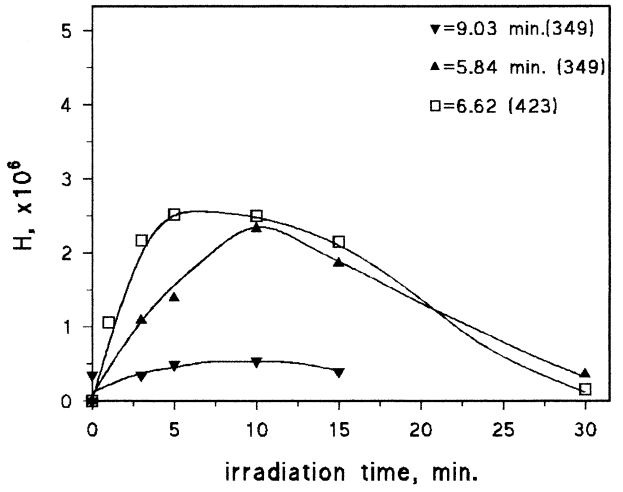


Figure 7. Formation of some intermediates holding *m/z* 423 and 349 from dexamethasone (1.3×10^{-4}M) degradation on TiO_2 200 mgL^{-1}.

The successive degradation step is the loss of the acetate group in part A of the species at *m/z* 423 to give the molecule at *m/z* 349 (Figure 7). The cleavage of the lateral chain was also found to be a metabolic product of dexamethasone in liver [1–3]. In particular, the structure labeled **19** in Scheme **1** has been recognized in liver. The MS/MS analysis is illustrated in Table 5. With regard to the difference in intensity of the same *m/z* values for the various species, similar considerations visible above for analogous mass spectrometric behaviors are valid.

As can be seen from data reported in Table 5, parallel to the fragmentation resulting from HF and H_2O losses, a ring cleavage is observed. The fragment 265 is obtained from 293 through a CO loss, from which an ethylene molecule has been released (fragment 237). This is observable in all the presented spectra. A concomitant fragmentation leads from *m/z* 311 to 283 through a similar bond cleavage and a CO loss.

The degradation pathways described in Scheme **1** represent only one of the possible transformation routes. In Figure 3 there is evidence of the presence of several species at *m/z* 425. Their kinetic evolution is comparable to that of the species at *m/z* 409 as can be seen by comparing Figures 8 and 4, thereby suggesting the existence of a concomitant pathway.

The difference in aμ units with respect to the parent molecule corresponds numerically to a double ·OH radical substitution. On the basis of the MS/MS analysis (Table 6) we have assumed the formation of the species represented in Scheme **2**. From the MS/MS analysis there is evidence of the loss of HF and two H_2O molecules in all cases. So we cannot hypothesize, as done before, that ·OH radical attacks methylene groups because in such a case we should have evidence of the loss of more than two H_2O molecules. We are then forced to conclude that the radical attacks on the double bonds of the quinoid moiety of the molecule. At this point the more probable hypothesis is in favor of the attack of two radical ·OH on the double bonds of the quinoid moiety.

Moreover, the MS/MS spectra indicate the presence

Table 4. Fragmentation obtained from MS/MS spectra of species holding *m/z* 423 ($C_{22}H_{27}O_7F$)

Fragment	Possible structure	Associated loss	1. t_R 3.51 min, intensity	2. t_R 3.83 min, intensity	3. t_R 4.20 min, intensity	4. t_R 6.05 min, intensity	5. t_R 10.4 min, intensity
405	$C_{22}H_{25}O_6F$	H_2O	100	100	100	100	80
403	$C_{22}H_{26}O_7$	HF	15	15	48	16	5
387	$C_{22}H_{23}O_5F$	H_2O	38	48	68	60	-
385	$C_{22}H_{24}O_6$	HF	40	60	58	-	30
369	$C_{22}H_{21}O_4F$	H_2O	32	-	-	18	-
367	$C_{22}H_{22}O_4$	HF	15	40	51	-	12
359	$C_{21}H_{26}O_5$	CO_2	40	28	5	15	-

Table 5. Fragmentation obtained from MS/MS spectra of species holding *m/z* 349 ($C_{20}H_{25}O_4F$)

Fragment	Possible structure	Associated loss	1. t_R 4.85 min, intensity	2. t_R 5.77 min, intensity	3. t_R 7.34 min, intensity	4. t_R 7.81 min, intensity	5. t_R 9.7 min, intensity
329	$C_{20}H_{24}O_4$	HF	87	100	32	25	98
311	$C_{20}H_{22}O_3$	H_2O	100	45	100	100	100
293	$C_{20}H_{20}O_2$	H_2O	45	18	70	46	71
275	$C_{20}H_{18}O$	H_2O	9	32	-	-	38
283	$C_{19}H_{22}O_2$	CO	7	35	10	380	67
265	$C_{19}H_{20}O$	CO	14	7	8	18	16
237	$C_{17}H_{16}O$	C_2H_4	5	5	5	18	16

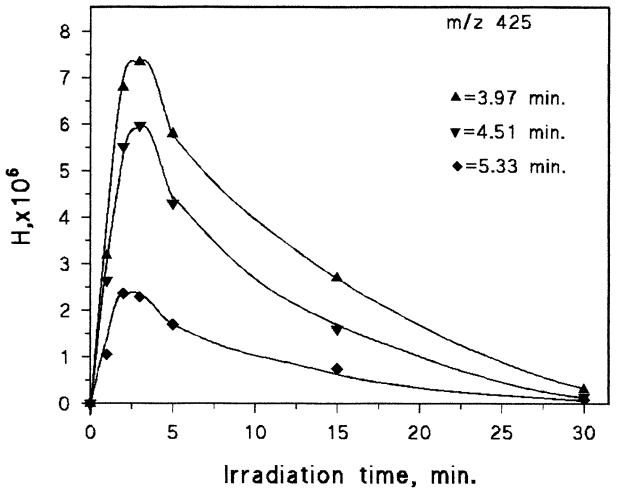


Figure 8. Formation of intermediates from dexamethasone (1.3×10^{-4}M) degradation on TiO_2 200 mgL^{-1}; *m/z* 425.

of peculiar daughter ions for the fragmentation patterns A, B, and C (*m/z* 353 and 333 for cases A and B and *m/z* 307 and 327 for case C). Such ions can be interpreted to be a consequence of the hydroxylation on the double bonds. In fact, the presence of several OH groups on the ring leads to unstable molecules and allows for new fragmentation pathways otherwise impossible for the species examined so far. Figure 9 illustrates such fragmentation involving the quinoid ion moiety. In case A (and similarly in the case B) the ion of *m/z* 353 derives from the loss from the parent ion (*m/z* 425) of the neutral molecule of mass 72 aμ, while the *m/z* 333 comes from *m/z* 353 and corresponds to a further loss of HF. A pathway of this type can be followed by species 1 and 2 represented in Scheme **2** and corresponds to the MS/MS spectra A and B.

In case C, the ion of *m/z* 327 derives from the loss of the same parent ion of the 98 aμ neutral molecule, while the *m/z* 307 is due to a further loss of HF. It forms species 3 represented in Scheme **2**. Also, in this case the lost molecule having *m/z* 98 derives from the breaking up of the quinoic ring and aids in the formation of a stable molecule. In the structure in case **C**, after the breaking up of the ring, a release of two molecules of water (*m/z* 289 and 271) can be observed. These losses also occur in the structures in cases of A and B, even if

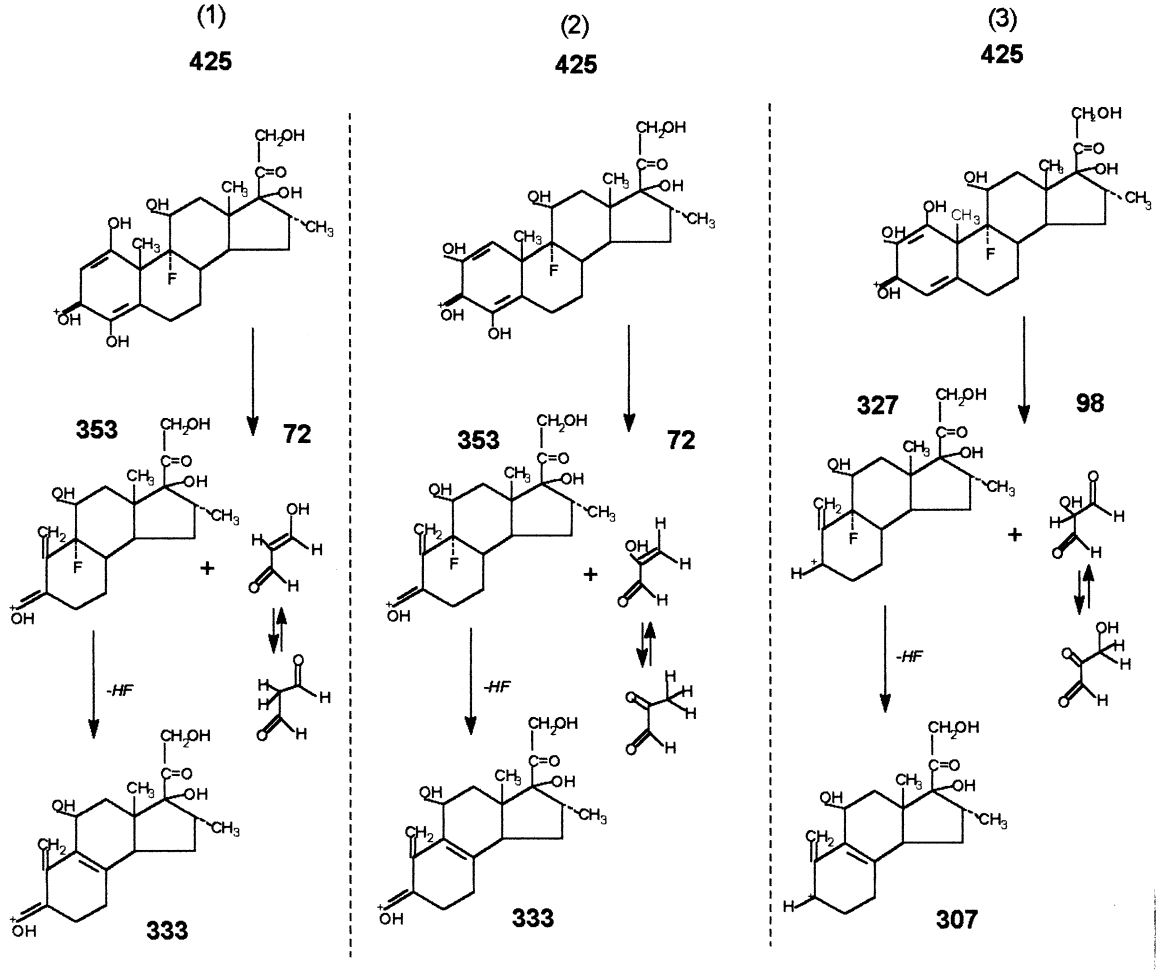


Figure 9. Fragmentation scheme for the different species having *m/z* 425.

Table 6. Fragmentation obtained from MS/MS spectra of species holding *m/z* 425 ($C_{22}H_{29}O_7F$)

Fragment	Possible structure	Associated loss	A. t_R 3.92 min, intensity	B. t_R 4.0 min, intensity	C. t_R 5.4 min, intensity
407	$C_{22}H_{27}O_6F$	H_2O	100	100	33
405	$C_{22}H_{28}O_7$	HF	56	61	-
387	$C_{22}H_{26}O_6$	H_2O	45	24	100
369	$C_{22}H_{24}O_5$	H_2O	14	14	40
353	$C_{19}H_{25}O_5F$	$C_3H_4O_2$	36	30	-
333	$C_{19}H_{24}O_5$	HF	34	11	-
315	$C_{19}H_{22}O_4$	H_2O	20	11	-
297	$C_{19}H_{20}O_3$	H_2O	5	-	-
327	$C_{19}H_{25}O_4F$	$C_3H_4O_3$	-	-	22
307	$C_{19}H_{24}O_4$	HF	-	-	36
289	$C_{19}H_{22}O_3$	H_2O	-	-	18
271	$C_{19}H_{20}O_2$	H_2O	-	-	16

these fragments are minimal. In case B only the first loss of H_2O can be appreciated.

Even if oxidation of the intermediates goes on leading to further degradation species, we will not discuss it here.

Conclusions

The photoinduced transformation of dexamethasone is basically oxidative in type and is initiated by ·OH radical attacks. More than one mechanism is present and we have obtained significant evidence of the presence of a hydroxylation mechanism involving both the methylene groups of the molecule and the quinoid ring. In this second case the fragmentation pattern shows molecular losses leading to the opening of the ring structure, evidencing a weakening of the molecular stability. It is likely that this degradation pathway is preferred for a more rapid degradation of the molecule.

The main goal of this study was to artificially produce degradation intermediates of dexamethasone which could be considered reasonably similar to those really present in the metabolic system of living organisms. It is known that hydroxylation is one of the principal metabolic ways operating in the liver. The structures found at present in liver studies have also been identified adopting a photocatalytic method, so that some of the molecules presented in this work can probably also be successfully found in bovine urine during veterinary controls. Investigations in this direction are in progress in our laboratory.

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